RESEARCH NOTE

Evaluation of the use of RT-PCR for the early diagnosis of dengue fever

M. P. Grobusch[1], M. Niedrig[2], K. Göbels[1], K. Klipstein-Grobusch[3] and D. Teichmann[1]

[1]Medical Clinic, Department of Infectious Diseases, Charité/Campus Virchow Hospital, Humboldt University, [2]Robert Koch Institute, Berlin, and [3]German Institute of Human Nutrition (DIfE), Nuthetal, Germany

ABSTRACT

RT-PCR was used to diagnose dengue virus infections confirmed serologically in 26 returning travellers. RT-PCR was positive for three (75%) of four samples taken on or before day 3 of the illness, for 15 (78.9%) of 19 samples taken between days 4 and 7, and for none of three samples tested on or after day 8 (p 0.0337). When applied early, RT-PCR seems to be a useful tool for the diagnosis of dengue fever.

Keywords Dengue fever, diagnosis, RT-PCR, sampling time, sensitivity

Original Submission: 16 June 2005; **Revised Submission:** 17 August 2005; **Accepted:** 14 September 2005

Clin Microbiol Infect 2006; 12: 395–397
10.1111/j.1469-0691.2006.01353.x

Dengue fever (DF) is among the most important arthropod-borne virus diseases in terms of human morbidity and mortality. The spectrum of this disease, caused by the four distinct dengue virus serotypes, DEN-1 to DEN-4, ranges from a self-limiting febrile illness (DF) to dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) [1]. With *c.* 2.5 billion people living in endemic areas, there are 50–100 million cases of dengue fever and 250 000–500 000 cases of DHF/DS with 24 000 deaths, annually [2]. This disease has become increasingly important in the differential diagnosis of fever of unknown origin in returning travellers [3–5].

Clinical observation is the key to case identification, but diagnosis by virus-specific antibody determination is crucial for confirmation and differentiation among the different dengue virus serotypes [1]. The existence of cross-reactive antigenic determinants shared by all four dengue virus serotypes and other flaviviruses means that the most precise serological diagnosis that can be offered by many laboratories is 'acute flavivirus infection', rather than 'acute dengue infection' [1]. It has been shown that direct detection and typing of dengue virus in serum using RT-PCR or culturing techniques yields the most reliable direct evidence of infection. The present study examined the period during the course of illness when RT-PCR could be used successfully to detect virus in a cohort of travellers presenting with dengue fever.

The study investigated 26 patients with serologically confirmed dengue fever who presented during a 6-year period at the Department of Infectious Diseases, Charité University Hospital, Berlin, Germany. Laboratory diagnosis of dengue fever was performed at the Robert Koch Institute, Berlin, Germany, with virus isolation in cell culture on the insect cell line C6/36, followed by immunofluorescence testing for virus antigens with dengue virus (DEN-1, -2, -3 and -4) type-specific monoclonal antibodies [1]. Additionally, a flavivirus type-specific RT-PCR, followed by a semi-nested PCR with primers specific for dengue virus strains 1–4, was performed [6]. Day 1 was considered to be the day of onset of disease, which is usually fever [5]. Age, gender and travel history (i.e., dengue-endemic area visited) were recorded for each patient. Statistical analysis was performed with SAS v.9.1 software (SAS Institute, Cary, NC, USA).

Of the 26 patients included in the study, 12 were female (mean age 31.3 years) and 14 were male (mean age 35.4 years). Travel destinations were South-east Asia (Thailand, $n = 14$; Indonesia, $n = 1$; Philippines, $n = 1$), the Indian subcontinent (India, $n = 4$; Sri Lanka, $n = 1$), Central America (El Salvador, $n = 1$; Nicaragua, $n = 1$), South America (Venezuela/Colombia, $n = 1$), Pacific Islands (Cook Islands, $n = 1$) and West

Corresponding author and reprint requests (current affiliation): M. P. Grobusch, Division of Clinical Microbiology and Infectious Diseases, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2196, Johannesburg, South Africa
E-mail: grobuschmp@pathology.wits.ac.za

Table 1. Detection of dengue virus RNA by RT-PCR at various time-points in the illness

Day of illness[a]	RT-PCR results[b]	p[c]
< 4	3/4 (75.0%)	
4–7	15/19 (78.9%)	0.0337
> 7	0/3 (0%)	

[a]Time-point of testing was predominantly identical with day of presentation.
[b]No. of positive samples tested/total no. of samples.
[c]Fisher's exact test.

Africa (Ghana, $n = 1$). Diagnosis of dengue fever was confirmed serologically by paired sera [1] in all cases. Table 1 summarises the RT-PCR results, grouped according to the day of illness when the diagnostic specimen was obtained. Of 18 positive RT-PCR results, eight were positive for DEN-1, five for DEN-2, and five for DEN-3. Additional virus isolation was successful and confirmatory for all eight cases positive for DEN-1, and for one case positive for DEN-2, but for none of the cases positive for DEN-3. Virus isolation was unsuccessful for all eight serologically confirmed cases that were negative by RT-PCR. In 19 (73.1%) cases, the initial serum sample was negative for dengue-specific IgM antibody, but timely confirmation of diagnosis was made by RT-PCR in 15 (78.9%) of these 19 cases.

Dengue virus infection should be considered if the clinical signs, and geographical and epidemiological circumstances, fit with a possible incubation period of 5–7 days. IgM formation in primary dengue infections usually increases markedly around day 4 or 5 of the illness [7]. An increasing immune response corresponds with a decreasing virus load, and there was a statistically significant difference (p 0.0337) when the results of RT-PCR were compared after <4, 4–7 and >7 days of illness (Table 1).

These cases of imported dengue fever highlight the importance of including RT-PCR in early diagnostic procedures for travellers returning from dengue endemic areas, particularly when initial serological tests, as in the majority of cases, show a minimal antibody response. Direct detection and typing of dengue virus in serum by RT-PCR is currently the fastest diagnostic technique for confirming clinical suspicion, while serotyping can provide vital information concerning patients with secondary dengue virus infection who are at a higher risk of developing DHF.

Whereas the current data support the diagnostic use of RT-PCR in the early phase of the disease, the method has several limitations. Most importantly, the period during which dengue virus can be detected successfully in serum samples is brief, and RT-PCR is not available in most clinical microbiology laboratories. This approach cannot provide live virus for further biological characterisation, and its sensitivity appears to vary between serotypes and protocols [8]. However, although isolation and typing of dengue virus might be considered to be the reference standard, it also has a small window of opportunity for detection [1], and modified RT-PCR protocols have yielded better results than virus isolation in some settings [9]. With the possibility of using quantitative RT-PCR to estimate the risk of an individual progressing towards DHF, the role of this technique may increase in the future [10]. In the meantime, as demonstrated by molecular proficiency tests [11,12], technical deficiencies in individual laboratories must be identified and eliminated. In the future, the use of molecular techniques for the diagnosis of imported dengue virus infections should enable clinicians and researchers to obtain a more precise picture of the disease's endemicity [13], thus facilitating a better understanding of its epidemiology and control.

REFERENCES

1. Teichmann D, Göbels K, Niedrig M, Sim-Brandenburg JW, L'age-Stehr J, Grobusch MP. Virus isolation for diagnosing dengue virus infections in returning travelers. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 697–700.
2. Mairuhu AT, Wagenaar J, Brandjes DP, van Gorp EC. Dengue: an arthropod-borne disease of global importance. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 425–443.
3. Wichmann O, Mühlberger N, Jelinek T. Dengue – the underestimated risk in travellers. *Dengue Bull* 2003; **27**: 126–137.
4. Jelinek T, Muhlberger N, Harms G *et al*. Epidemiology and clinical features of imported dengue fever in Europe: sentinel surveillance data from TropNetEurop. *Clin Infect Dis* 2002; **35**: 1047–1052.
5. Teichmann D, Göbels K, Niedrig M, Grobusch MP. Dengue virus infection in travellers returning to Berlin, Germany: clinical, laboratory, and diagnostic aspects. *Acta Tropica* 2004; **90**: 87–95.
6. ter Meulen J, Grau M, Lenz O *et al*. Isolation and partial characterisation of dengue virus type 2 and 4 strains from dengue fever and dengue haemorrhagic fever patients from Mindanao, Republic of the Philippines. *Trop Med Int Health* 2000; **5**: 325–329.

7. Vaughn DW, Green S, Kalayanarooj S *et al.* Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis* 1997; **176**: 322–330.
8. Sudiro TM, Ishiko H, Green S *et al.* Rapid diagnosis of dengue viremia by reverse transcriptase-polymerase chain reaction using 3'-noncoding region universal primers. *Am J Trop Med Hyg* 1997; **56**: 424–429.
9. De Paula SO, Pires Neto RJ, Correa JA *et al.* The use of reverse transcription-polymerase chain reaction (RT-PCR) for the rapid detection and identification of dengue virus in an endemic region: a validation study. *Trans R Soc Trop Med Hyg* 2002; **96**: 266–269.
10. Wang WK, Chao DY, Kao CL *et al.* High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. *Virology* 2003; **305**: 330–338.
11. Donoso Mantke O, Lemmer K, Biel SS *et al.* Quality control measures for the serological diagnosis of dengue virus infections. *J Clin Virol* 2004; **29**: 105–112.
12. Lemmer K, Donoso Mantke O, Bae HG, Groen J, Drosten C, Niedrig M. External quality control assessment in PCR diagnostics of dengue virus infections. *J Clin Virol* 2004; **30**: 291–296.
13. Domingo C, Palacios G, Niedrig M *et al.* A new tool for the diagnostic and molecular surveillance of Dengue infections in clinical samples. *WHO Dengue Bull* 2005; **28**: 87–95.

RESEARCH NOTE

Antifungal activity of antimicrobial-impregnated devices

R. O. Darouiche, M. D. Mansouri and E. M. Kojic

Section of Infectious Diseases, Veterans Affairs Medical Center and Center for Prostheses Infection, Baylor College of Medicine, Houston, TX, USA

ABSTRACT

The in-vitro and in-vivo efficacy against *Candida albicans* and *Candida krusei* of devices impregnated with chlorhexidine and chloroxylenol was examined. The impregnated devices produced large zones of inhibition against both organisms (mean size, 39 mm and 38 mm, respectively). In a rabbit model in which segments of silicone catheters were placed percutaneously, non-impregnated devices were twice as likely as impregnated devices to become colonised with either *C. albicans* or *C. krusei*. Impregnated devices also had significantly lower colony counts of *C. albicans* (58 vs. 1361 CFU; p 0.008) and *C. krusei* (19 vs. 764 CFU; p 0.008).

Corresponding author and reprint requests: R. O. Darouiche, Center for Prostheses Infection, Baylor College of Medicine, 1333 Moursund Avenue, Suite A221, Houston, TX 77030, USA
E-mail: rdarouiche@aol.com

Keywords *Candida albicans*, *Candida krusei*, chlorhexidine, chloroxylenol, impregnated devices, rabbit model

Original Submission: 18 May 2005; **Revised Submission:** 23 August 2005; **Accepted:** 21 September 2005

Clin Microbiol Infect 2006; 12: 397–399
10.1111/j.1469-0691.2006.01369.x

Candida spp. are recognised increasingly as important causes of infection associated with medical devices [1]. Recent surveillance data from 49 hospitals indicated that *Candida* spp. cause 9% of hospital-acquired bloodstream infections [2]. *Candida* infections associated with medical devices tend to be more problematic than bacterial infections. Thus, *Candida* continues to be associated with the highest crude mortality rate (39–40%) resulting from hospital-acquired bloodstream infections [2,3].

The serious medical complications, difficult management and high economic burden of *Candida* device-associated infections prompted an exploration of the potential preventive efficacy of innovative approaches [4]. Since device colonisation can be a prelude to clinical infection [5], it is possible that devices impregnated with agents that possess antifungal activity may be clinically protective. The objective of the present study was to investigate the in-vitro and in-vivo efficacy of devices impregnated with the antiseptic combination of chlorhexidine and chloroxylenol against *Candida albicans* (the most common *Candida* sp.) and *Candida krusei* (the most inherently azole-resistant *Candida* sp.).

Eighteen French silicone catheters (Cook Inc., Bloomington, IN, USA) were impregnated by dipping in a solution of chlorhexidine (50 mg/mL) and chloroxylenol (50 mg/mL), followed by drying overnight and rinsing with deionised water. Segments (2 cm) of both impregnated and control catheters were sterilised with ethylene oxide gas before testing. Mean quantities of 102 μg of chlorhexidine and 156 μg of chloroxylenol were extracted from 2-cm segments of the gas-sterilised impregnated catheters.